Supporting Information for:

Multi-Institution Research and Education Collaboration Identifies New Antimicrobial Compounds

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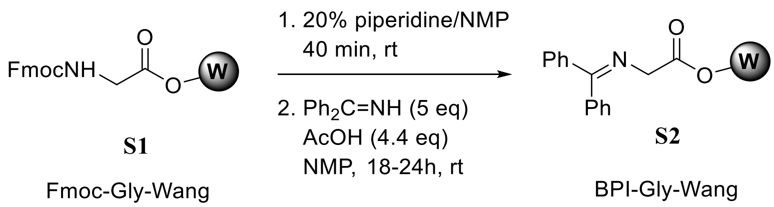
Sources of materials used in these studies

Resins were purchased from Peptides International (Louisville, KY), Advanced Chemtech (Louisville, KY) or EMD Millipore (Burlington, MA). All reagents were purchased from Acros (Geel, Belgium), Sigma-Aldrich (St. Louis, MO), or Advanced Chemtech and were used without further purification. Solid-phase peptide synthesis (SPPS) vessels were purchased from Chemglass (Vineland, NJ).

Preparation of the benzophenone imine of glycine on Wang resin (BPI-Gly-Wang, S2)[1]

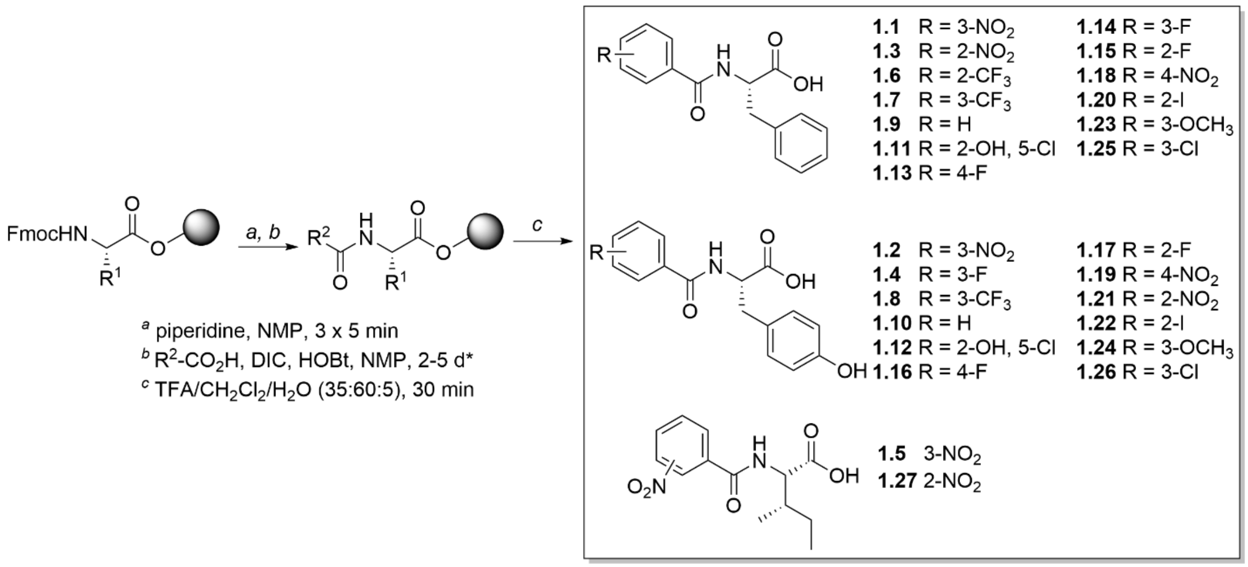


A 25-mL solid-phase peptide synthesis vessel was charged with 390 mg (300 μmoles) of Fmoc-Gly-Wang resin (**S1,** EMD Millipore 856009) and swelled in 5 mL of NMP for 30 min under argon.[1] The vessel was drained using argon[1,2] pressure and the resin was then washed with 2 × 8 mL of NMP. To the swelled, washed resin was added 3 mL of 20% (v/v) piperidine in NMP. After a 2-3 min exposure, the vessel was drained under argon[2,3] and the resin was again treated for 2-3 min with 3 mL of 20% piperidine in NMP. The vessel was drained and the resin was treated a third time with 10 mL of 20% piperidine in NMP. The vessel was rocked on an orbital shaker for 40 min and was drained using argon[1,2] pressure. The resin was washed with 6 × 8 mL × 2 min with NMP. To the deprotected resin was then added, in one portion, a solution of 272 mg (252 μL, 1.5 mmol, 5 eq) benzophenone imine in 3 mL of NMP followed by one portion of a 3.0 mL solution of 75 μL (79 mg, 1.3 mmol, 4.4 eq) acetic acid in NMP. The vessel was then rocked on an orbital shaker overnight. The vessel was then drained and the resin washed with 6 × 8 mL × 2 min with NMP.

For immediate use of resin: The wet resin was quantitatively transferred using NMP to a beaker in which an isopycnic suspension was prepared (approx. 2/1 v/v NMP/DCM). The suspension was then dispensed equally using an Eppendorf repeater pipet into six 3.5-mL reaction vessels held in a 2 × 3 Bill-Board and washed with 4 × 2 mL of NMP.

For delayed use of resin (storage): The wet resin was washed with 6 × 8 mL × 2 min with DCM and was dried in the SPPS vessel under a slow stream of dry nitrogen gas over 24 h and was stored under dry argon at 2-8 °C.

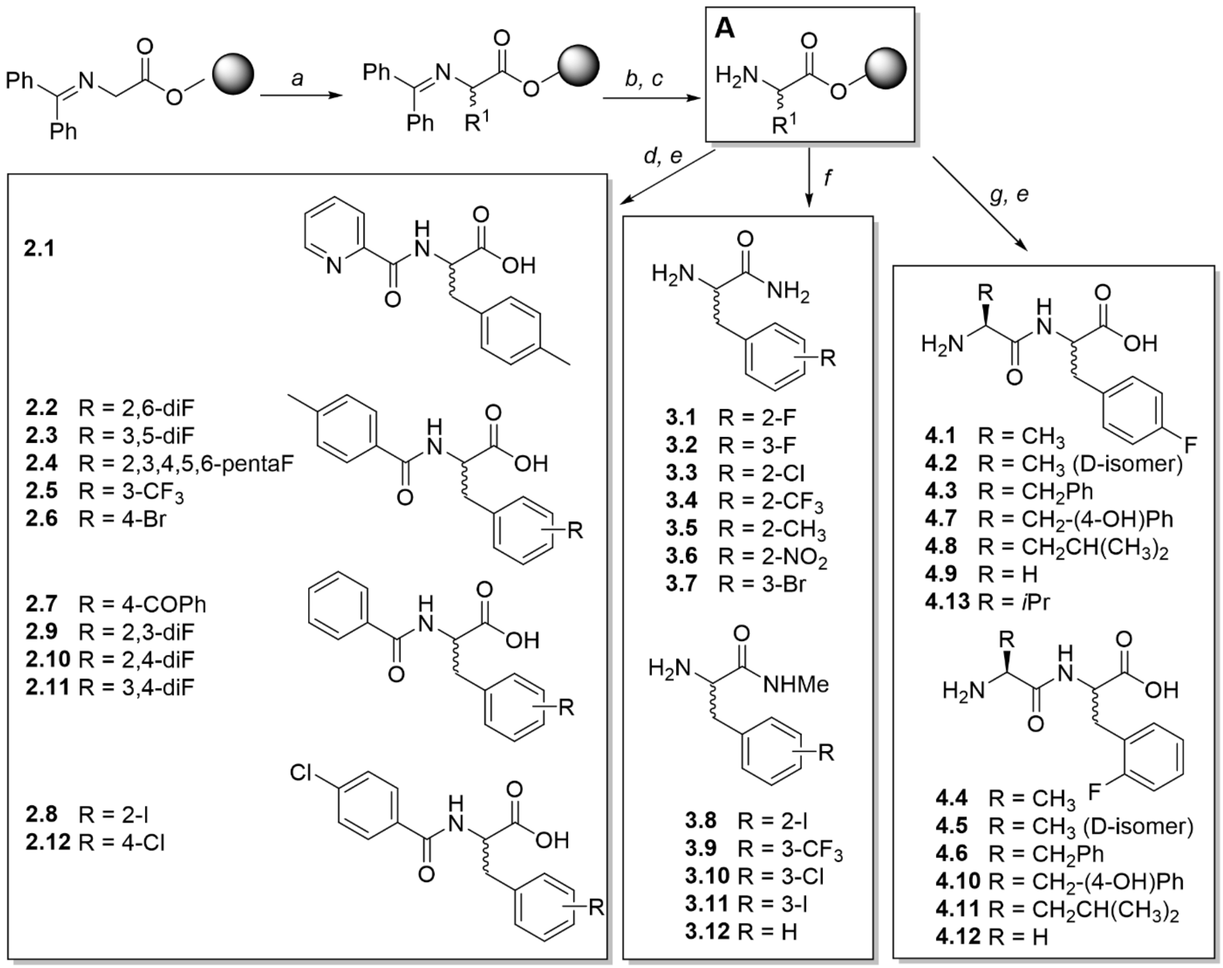
[1] JACS **1996**, *118*, 6070; TL **1997**, *38*, 7163
[2] Argon (or nitrogen) not necessary if lab environment is not humid.
[3] Draining will be slow without added pressure of inert gas or air.



Synthesis of *N*-acylated natural amino acids (1.1 – 1.27)

To each synthesis vessel in the Bill Board was added Wang resin pre-functionalized with the appropriate Fmoc-protected amino acid (50 µmol). The resin was washed three times with NMP, then vessels were capped at the bottom. To each reaction vessel, 2 mL of a 20% (v/v) solution of piperidine in NMP was added, and the resin was allowed to sit for 5 min. The reaction vessels were uncapped, drained, then re-capped and re-treated with fresh piperidine solution twice more. Following the third treatment, the reaction vessels were uncapped, drained, and washed three times with NMP, then capped on the bottom again. A solution was prepared of the appropriate acid (0.25 M solution in 0.25 M HOBt in NMP). To each reaction vessel, 1 mL of the appropriate solution was added (0.25 mmol acid, 5 equiv acid, 0.25 mmol HOBt, 5 equiv HOBt) followed by 0.5 mL of DIC solution (0.5 M in NMP, 0.25 mmol, 5 equiv). The tops of the reaction vessels were capped, and the Bill-Board was allowed to rotate or to sit after brief manual agitation for 2-5 days. The reaction vessels were uncapped, and each was washed twice with NMP, twice with THF, and three times with CH_2Cl_2.

To execute cleavage of the product from the resin, two methods have been used with comparable success. *Method A:* This method has the advantage that student researchers minimally handle reaction vessels charged with the strong acid solution because reaction vessel caps need not be removed. The resins in the *uncapped* reaction vessels were treated with 2 mL of a mixture of $TFA/CH_2Cl_2/H_2O$ (35/60/5) for 30 min allowing the filtrate to collect in separate vials from each reaction vessel. Each reaction vessel was rinsed into the collection vials with an additional 2 mL of the TFA reagent and then with 2 mL of CH_2Cl_2. *Method B:* The resins in *capped* vessels were treated with 2 mL of $TFA/CH_2Cl_2/H_2O$ (35/60/5) for 30 min. The reaction vessel caps were then removed, and the solution was collected into individually labeled, tared vials. Each reaction vessel was then rinsed with an additional 2 mL of each $TFA/CH_2Cl_2/H_2O$ (35/60/5) and CH_2Cl_2. Following either cleavage method, a small aliquot of the filtrate (100 µL) was evaporated to dryness and was analyzed by liquid chromatography-mass spectrometry (LC/MS). The remainder was concentrated by evaporation under a stream of N_2 (effluent TFA vapor was scrubbed in a caustic solution of sodium hydroxide).



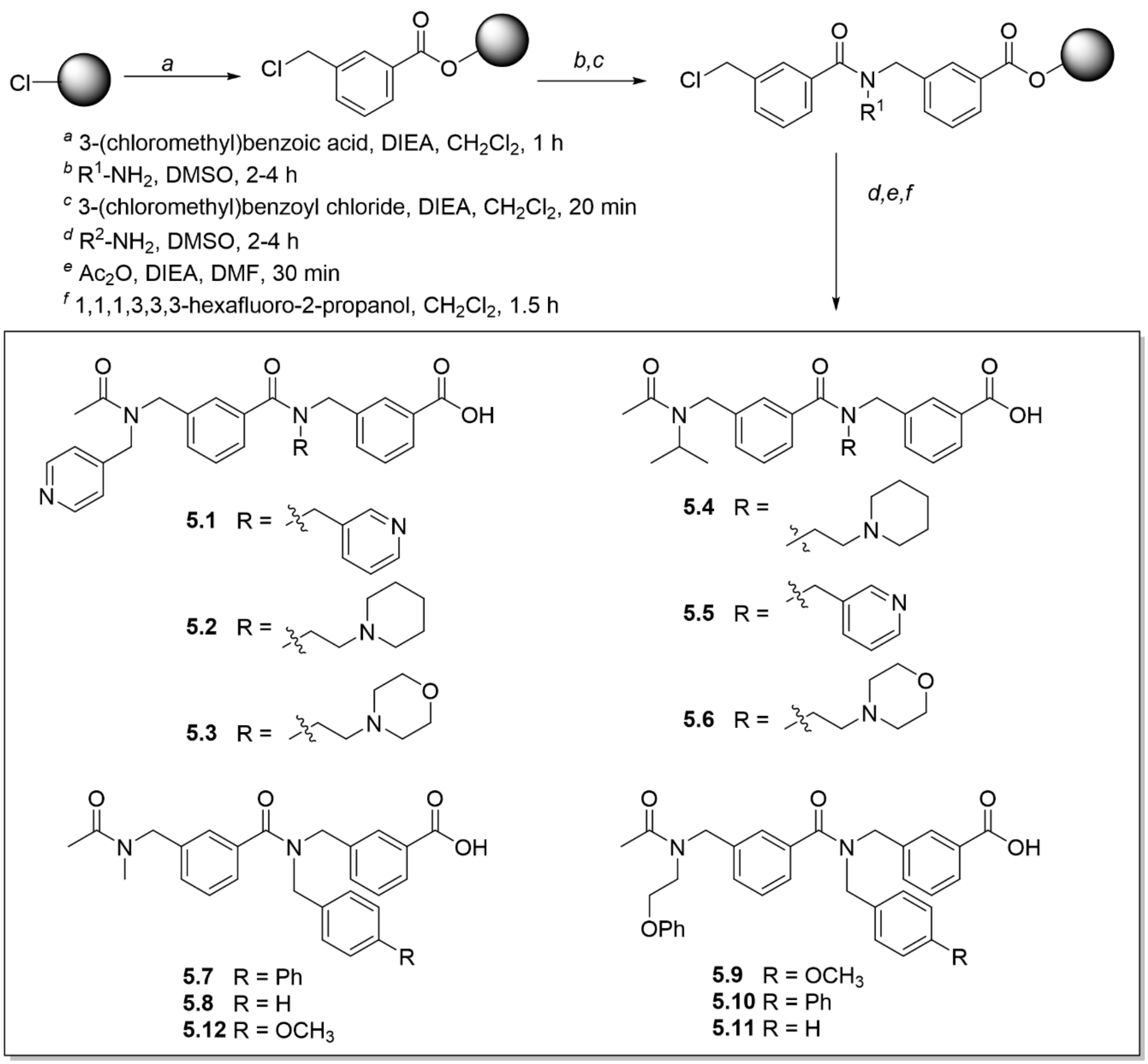
[a] R^1X, *t*-butylimino-tri(pyrrolidino)phosphorane, NMP, 2 d; [b] 1 N HCl/THF (1:2), 20 min; [c] DIEA; [d] R^2-COOH, DIC, HOBt, NMP, 2 d; [e] TFA/CH_2Cl_2/H_2O (35:60:5), 30 min; [f] R^2-NH_2, MeOH or THF, 12 d*; [g] N-BOC-amino acid, DIC, HOBt, NMP, 2-5 d*

Synthesis of unnatural amino acid intermediate A

Each synthesis vessel was loaded with benzophenone imine of glycine-Wang resin (0.05 mmol) and the resin was washed 3 x 3 mL with NMP. The reaction vessels were capped at the bottom, and to each was added *tert*-butylimino-tri(pyrrolidino)phosphorene (BTTP, 0.5 mL of a 0.2 M solution in NMP, 0.1 mmol, 2 equiv). The reaction mixture was allowed to sit for 5 min, then a solution of the alkyl halide (0.5 mL of a 0.2 M solution in NMP, 0.1 mmol, 2 equiv) was added. The Bill-Board was inverted twice to ensure complete mixing, then allowed to sit at room temperature for 2-7 days. Following the reaction time, each reaction vessel was uncapped and washed with 3 mL THF. Bottom reaction vessel caps were replaced, and to each reaction vessel was added 2.5 mL of a 1 N aqueous HCl/THF (1:2) solution. The reaction vessels were capped, and the Bill-Board was inverted twice to ensure complete mixing, then allowed to stand for 20 min. Upon completion of the reaction, the reaction vessels were uncapped, then rinsed 1x 3 mL with THF, 2 x 2.5 mL with a 0.20 M solution of DIEA in NMP, and 2 x 2.5 mL with NMP.

Synthesis of *N*-acylated unnatural amino acids (2.1 – 2.12)

From intermediate **A**, the reaction vessels were capped at the bottoms. A solution was prepared of the appropriate acid (0.25 M solution in 0.25 M HOBt in NMP). To each reaction vessel, 1 mL of the appropriate solution was added (0.25 mmol acid, 5 equiv acid, 0.25 mmol HOBt, 5 equiv HOBt) followed by 0.5 mL of DIC solution (0.5 M in NMP, 0.25 mmol, 5 equiv). The tops of the reaction vessels were capped, and the Bill Board was allowed to rotate or sit undisturbed after manual agitation for 2-5 days. The reaction vessels were uncapped, and each was washed twice with NMP, twice with THF, and thrice with CH_2Cl_2. Reaction vessels were capped at the bottom, then each was treated with 2 mL of a mixture of TFA/ CH_2Cl_2/H_2O for 30 min. The reaction vessel caps were then removed, and the solution was collected into individually labeled, tared vials. Each reaction vessel was rinsed with an additional 2 mL of the cleavage solution, then 2 mL CH_2Cl_2 (Method B). A small aliquot of the filtrate (100 μL) was evaporated to dryness and was analyzed by LC/MS. The remainder was concentrated by evaporation under a stream of N_2 (effluent TFA vapor was scrubbed in a caustic solution of sodium hydroxide).



Synthesis of arylopeptoids (5.1 – 5.12)

To each synthesis vessel in the Bill Board, 2-chlorotrityl chloride resin (0.025 mmol) was added. The resin was rinsed three times with CH_2Cl_2 and swelled in CH_2Cl_2 for at least ten minutes. The vessel was drained and capped at the bottom. A mixture of 3-(chloromethyl)benzoic acid (214 μL, 0.14 M in CH_2Cl_2, 0.03 mmol, 1.2 equiv) and DIEA (0.15 mmol, 6 equiv) was prepared and then immediately added to each reaction vessel. The reaction vessels were capped at their tops, then agitated by gently shaking the Bill Board occasionally over 1 h. The reaction vessel caps were removed from the bottom, then the top, and the vessels were drained, then rinsed thrice with CH_2Cl_2 then twice with DMSO, pushing remaining solvent out of the vessel between rinses with positive air pressure. The bottom reaction vessel caps were replaced, and a solution of the appropriate primary amine (250 μL of a 2 M solution in DMSO, 0.5 mmol, 20 equiv) was added to each reaction vessel. The reaction vessels were capped at their tops and the Bill Board was agitated gently periodically over 2-4 h. The vessel caps were removed, the resin was drained, and then rinsed 5 x with DMSO, then 2 x with CH_2Cl_2. A small number of resin beads were removed from each vessel and a chloranil test confirmed the presence of a secondary amine in each with a blue/green bead color. A mixture of 3-(chloromethyl)benzoyl chloride (150 μL, 0.5 M in CH_2Cl_2, 0.075 mmol, 3 equiv) and DIEA (0.15 mmol, 6 equiv) was prepared and then immediately added to each reaction vessel. The tops of the vessels were capped, and the Bill Board was agitated a few times over the 20 minute reaction time. The bottom caps, then top caps to the reaction vessels were removed, and the vessels were drained, then washed thrice with CH_2Cl_2 then twice with DMSO. A few beads from each vessel were removed and subjected to the chloranil test to confirm reaction completion (colorless beads). The reaction vessels were capped again at the bottom, and a solution of the appropriate amine (250 μL of a 2 M solution in DMSO, 0.5 mmol, 20 equiv) was added to each vessel. The reaction vessel tops were capped, and the Bill Board was agitated occasionally over the 2-4 h reaction time. In the case of methylamine addition at this step (analogs **5.7**, **5.8**, and **5.12**), the amine was added as a 33 wt% solution in absolute ethanol. The vessel caps were removed, the resin was drained and then rinsed 5 x with DMSO. A small number of resin beads were removed from each vessel and a chloranil test confirmed the presence of a secondary amine in each with a blue/green bead color. Finally, a solution of acetic anhydride (50 μL, 0.5 mmol, 20 equiv) and DIEA (0.17 mL, 1 mmol, 40 equiv) in 0.5 mL DMF was prepared and added to each reaction vessel. The vessels were capped and the Bill Board was gently agitated over the 30 min reaction time. The caps of the reaction vessels were removed, the vessels were drained, and then washed thrice with CH_2Cl_2. A chloranil test was again performed on a few beads from each vessel to confirm that the reaction was complete (colorless beads).

The reaction vessels were capped at the bottom, and crude arylopeptoids were subsequently cleaved from the resin by treatment of each reaction vessel with 0.5 mL of a 4:1 solution of 1,1,1,3,3,3-hexafluoro-2-propanol in CH_2Cl_2. After 90 min, the reaction vessel caps were removed, and the solution was collected into individually labeled, tared vials. Each reaction vessel was rinsed with an additional 0.5 mL CH_2Cl_2 and 0.5 mL methanol.

A small aliquot of the filtrate was subjected to LC/MS analysis. The remainder was concentrated by rotary evaporation, then dissolved in water and acetonitrile, frozen, and lyophilized. Further purification was effected by semi-preparative RP-HPLC on a Hitachi Chromaster 5000 instrument. Compounds were eluted from an AAPPTec Spirit Peptide C18 column (5 μM, 10.0 mm x 25 cm) using a linear gradient of methanol (solvent B) in 0.1% aqueous TFA (solvent A) at a flow rate of 3 mL/min flow rate. Peaks eluted were detected by absorbance at 220 nm, and data were visualized with EZChrom software.

BIOLOGICAL TESTING METHODS

Preliminary screening antimicrobial assay

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (CAMHB) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 °C for 1.5-3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD600), then added to each well of the compound containing plates, giving a cell density of 5-105 CFU/mL and a total volume of 50 µL. All the plates were covered and incubated at 37 °C for 18 h without shaking. Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD600), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references.

Preliminary screening antifungal assay

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 °C. A yeast suspension of 1×10^6 to 5×10^6 CFU/mL (as determined by OD530) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of 2.5 - 103 CFU/mL and a total volume of 50 µL. All plates were covered and incubated at 35 °C for 24 h without shaking. Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm (OD530), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm (OD600-570), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate.

Hit confirmation screening

Compounds identified as partially active or active in the preliminary screen were plated as a 2-fold dose response from 32 to 0.25 µg/mL, with a maximum of 0.5% DMSO, final in assay concentration. Growth inhibitions of all bacteria and fungi were measured as described in the preliminary screening methods. Compounds were classified as “active” if they exhibited MIC < 32 µg/mL against any of the pathogens tested. Compounds were classified as “cytotoxic” if they inhibited ≥ 50% of mammalian HEK293 cells at the highest concentration tested.

MIC determination

The minimum inhibitory concentration (MIC) was determined following the CLSI guidelines, identifying the lowest concentration at which full inhibition of the bacteria or fungi was detected. Full inhibition of growth has been defined at ≤ 20% growth, and concentrations were selected only if the next higher concentration displayed full inhibition as well.

Cytotoxicity assay

Cytotoxicity was assessed against human embryonic kidney cells (HEK293, ATCC CRL-1573), with data presented as CC50, corresponding to the concentration of the drug (µg/mL) at which 50% inhibition of cell viability is achieved. HEK293 cells were counted manually in a Neubauer hemocytometer and added to compound-containing plates (384-well plates, tissue culture treated

(TC); Corning CLS3712) giving a final density of 5000 cells/well in a total volume of 50 µL, using Dulbecco's Modified Eagle Medium (DMEM; Life Technologies 11995-073) with 10% Fetal Bovine Serum (FBS; GE SH30084.03). The cells were incubated together with the compounds for 20 h at 37 °C in 5% CO_2. Cytotoxicity (or cell viability) was measured by fluorescence, ex: 560/10 nm, em: 590/10 nm (F560/590), after addition of 5 µL of 25 µg/mL resazurin (2.3 µg/mL final concentration; Sigma R7017) and after further incubation for 3 h at 37 °C in 5% CO_2. Media only was used as negative control and cells without inhibitors as positive growth control. The fluorescence was measured using a Tecan M1000 Pro monochromator plate reader. CC50 (concentration at 50% cytotoxicity) values were calculated by curve fitting the inhibition values vs. log(concentration) using a sigmoidal dose-response function, with variable fitting values for bottom, top and slope. Tamoxifen (Sigma Aldrich, Australia T5648) was used as positive inhibitor control on each plate.

Table S1. Conditions for LC/MS analyses of crude reaction mixtures carried out by Lilly Analytical Technologies (IUPUI organic laboratory course student researcher samples), IUPUI (IUPUI and Goshen College research samples), Santa Clara University (Santa Clara research samples), Colorado College (Colorado College research samples).

Site	Instrument	Flow rate (mL/min)	solvents	Gradient	Column	Column temp.	UV detection	Mass spectrometry
IUPUI	Agilent Technologies 1200 and 1100 Series LC with 6130 Quadrupole MS	1.0	**A:** 5 mM ammonium acetate in water **B:** 5 mM ammonium acetate in 1:1 Acetonitrile:Methanol	20-100% B in 10.0 minutes with a 2.0 minute hold of 100% B	Agilent Eclipse XDB C18 (4.6 × 150 mm, 5-micron)	23 ºC	190-400 nm scan	ESI, positive mode (60-600 amu)
Santa Clara University	Dionex Ultimate 3000 LC-Thermo LCQ Fleet MS	0.5	**A:** 0.5% formic acid in water **B:** 0.5% formic acid in methanol	5-100% B for 5 min followed by a 2 min hold at 100% B, then re-equilibration for 3 min	Phenomenex Gemini C18 (50 x 2 mm, 5 micron)	N/A	254 nm	ESI, positive mode (150-2000 Da scan)
Colorado College	Waters Acquity UPLC/QDa Mass Spec	0.6	**A:** 5 mM ammonium acetate in water **B:** 5 mM ammonium acetate in 1:1 Acetonitrile:Methanol	5-95% B in 3.0 minutes with a 2.0 minute hold of 95% B	Acquity UPLC BEH C18, (2.1 x 50mm, 1.7 micron)	40 ºC	190-400 nm scan	ESI Negative Mode (200-450 Da Scan)
Lilly Analytical Technologies[a] (“QC_T0” method)	LC: Agilent 1290 Infinity 2: MS: Agilent 6150 single quadrupole	1.0	**A:** Water with 0.1% Formic Acid **B:** Acetonitrile with 0.1% Formic Acid	0.2 minute hold of 5% B, 5-100% B in 3.0 minutes with a 0.5 minute hold of 100% B	Phenomenex Gemini-NX C18 (2.00 x 50mm, 3 micron)	50 ºC	100-500 nm scan	Dual polarity scanning from 100-1300 AMU
Lilly Analytical Technologies[a] (“QC_T0_HIGH” method)		1.0	**A:** 95/5 Water/Acetonitrile with 10mM Ammonium Bicarbonate pH=10 **B:** Acetonitrile	0.2 minute hold at 5% B, 5-100% B in 3.0 minutes with a 0.5 minute hold of 100% B	Waters X-Bridge C18 (2.1 x 50mm, 3.5 micron)	50 ºC	100-500 nm scan	Dual polarity scanning from 100-1300 AMU
Lilly Analytical Technologies[a] (“QC_T0_ATL” method)		1.4	**A:** Water with 0.1% Formic Acid **B:** Acetonitrile with 0.1% Formic Acid	0.3 minute hold at 5% B, 1-100% B in 3.0 minutes with a 0.5 minute hold of 100% B	Waters Atlantis T3 (2.1 x 50mm, 3 micron)	50 ºC	100-500 nm scan	Dual polarity scanning from 100-1300 AMU

[a] Samples were received dried down to a weight of approximately 0.03 mg each. 10 μL of DMSO was added to each sample in order to achieve a concentration of approximately 10 mM for LC/MS analysis. For each sequence, standard compounds that vary in molecular weight, ionization, and retention time were run to ensure instrument functionality. Standards were run at the beginning and end of each group of samples, and approximately every 30 samples.

Table S2. Synthesis and biological screening summary for all compounds.

Synthesis data

Crude purity: determined by integration of LC/MS chromatogram. 1.11a and 1.11b crude purities were determined by quantitative NMR (QNMR) analysis[a]

Crude mass: (P) indicates mass of a sample following purification by chromatography, typically as part of the laboratory exercises; (QNMR) indicates value obtained by quantitative NMR analysis of the crude sample.[a]

Preliminary biological screening data

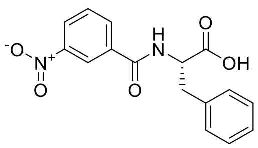
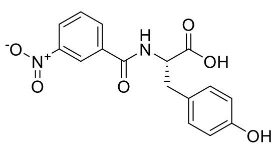
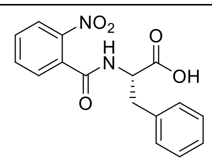
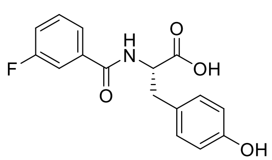
Sa: *Staphylococcus aureus*
Ec: *Escherichia coli*
Kp: *Klebsiella pneumoniae*
Pa: *Pseudomonas aeruginosa*
Ab: *Acinetobacter baumannii*
Ca: *Candida albicans*
Cn: *Cryptococcus neoformans*

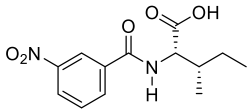
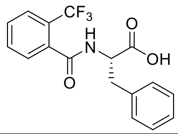
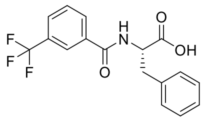
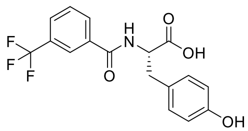
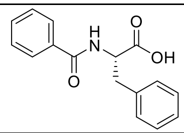
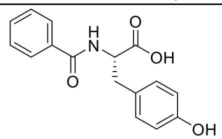
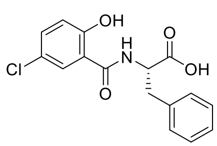
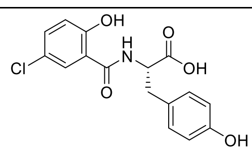
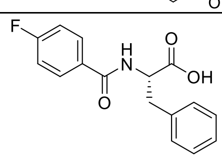
NA indicates < 50% growth inhibition
P indicates 50-80% growth inhibition
A indicates 80% growth inhibition

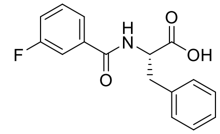
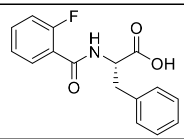
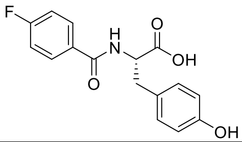
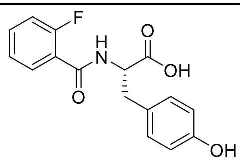
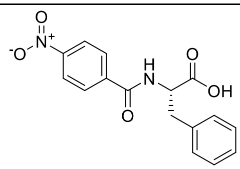
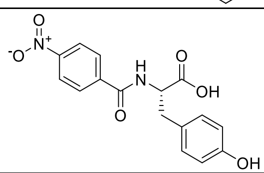
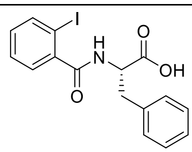
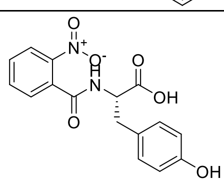
Hit confirmation biological screening

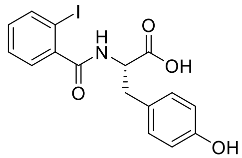
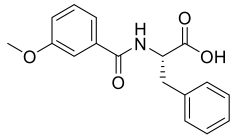
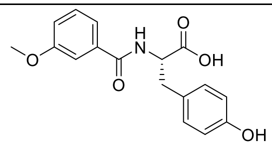
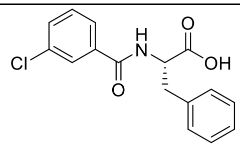
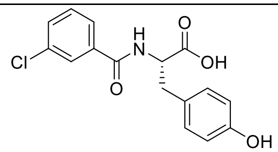
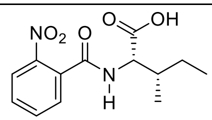
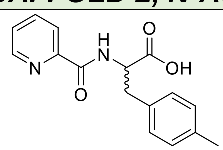
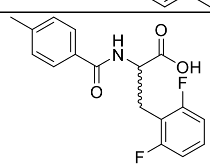
Active: blank cells indicate data were not collected in this screen; NO indicates that compounds exhibited MIC > 32 µg/mL for all of the pathogens; YES indicates MIC ≤ 32 µg/mL for any of the pathogens.

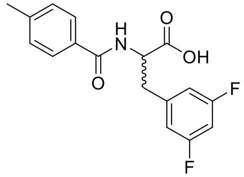
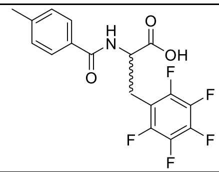
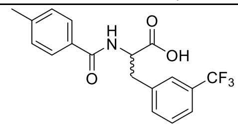
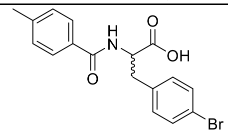
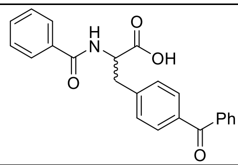
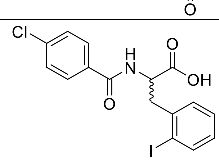
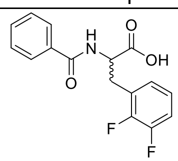
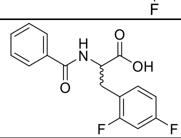
Cytotoxic: YES indicates ≥ 50% growth inhibition of HEK293 cells at the highest concentration tested for at least one replicate. NO indicates < 50% growth inhibition of HEK293 cells at the highest concentration tested.

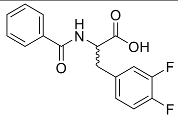
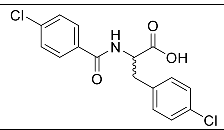
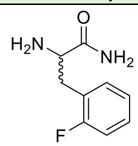
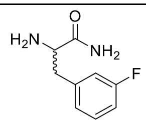
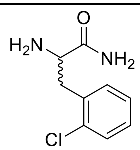
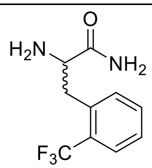
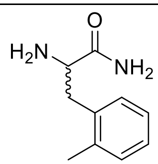
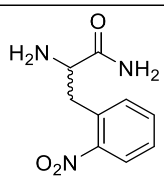
Structure	number	school	Crude purity	Crude mass (mg)	Sa	Ec	Kp	Pa	Ab	Ca	Cn	Active	Pathogen, MIC (µg/mL)	Cytotoxic
SCAFFOLD 1, N-Acylated natural amino acids														
	**1.1a**	Colorado College	100	4.0 (P)	NA	NA	NA	NA	NA	NA	NA			
	1.1b	IUPUI	100	17.7 12.0 (QNMR) 10.9 (P)	NA	NA	NA	NA	NA	NA	NA			
	1.1c	U. Havana	57	12.4	NA	NA	NA	NA	NA	NA	NA			
	1.1d	U. Havana	89	14.2	NA	NA	NA	NA	NA	NA	NA			
	**1.2a**	Colorado College	72	12.8	NA	NA	NA	NA	NA	NA	NA			
	1.2b	IUPUI	96	20.6 14.6 (QNMR) 12.2 (P)	NA	NA	NA	NA	NA	NA	NA			
	1.2c	U. Havana	15	11.2	NA	NA	NA	NA	NA	NA	NA			
	1.2d	U. Havana	92	11.5	NA	NA	NA	NA	NA	NA	NA			
	**1.3a**	Colorado College	67	3.4	NA	NA	NA	NA	NA	NA	NA			
	1.3b	Goshen College	100	10.6	NA	NA	NA	NA	NA	NA	NA			
	1.3c	U. Havana	91	17.2	NA	NA	NA	NA	NA	NA	NA			
	1.3d	U. Havana	51	15.4	NA	NA	NA	NA	NA	NA	NA			
	**1.4a**	Colorado College	54	2.6 (P)	NA	NA	NA	NA	NA	NA	NA			
	1.4b	IUPUI	100	17.3 11.4 (QNMR) 9.4 (P)	NA	NA	NA	NA	NA	NA	NA			
	1.4c	U. Havana	78	12.1	NA	NA	NA	NA	NA	NA	NA			
	1.4d	U. Havana	90	12.4	NA	NA	NA	NA	NA	NA	NA			

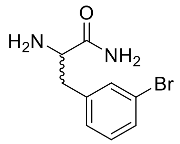
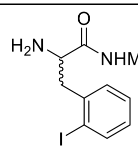
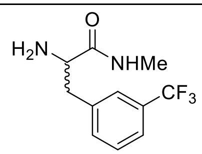
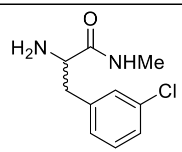
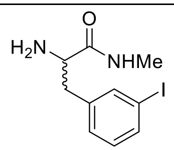
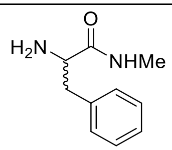
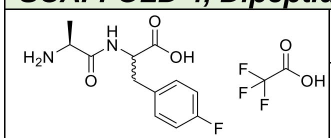
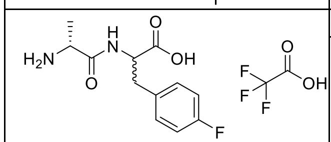
	**1.5**	Colorado College	69	10.5	NA	NA	NA	NA	NA	NA	NA			
	**1.6a**	Colorado College	97	3.7 (P)	NA	NA	NA	NA	NA	NA	NA			
	1.6b	IUPUI	94	21.8	NA	A	NA	NA	NA	NA	NA	NO		NO
	**1.7a**	Colorado College	98	4.5 (P)	NA	NA	NA	NA	NA	NA	NA			
	1.7b	IUPUI	95	20.5	NA	NA	NA	NA	NA	NA	NA			
	1.7c	U. Havana	63	14.6	NA	NA	NA	NA	NA	NA	NA			
	1.7d	U. Havana	67	13.9	NA	NA	NA	NA	NA	NA	NA			
	**1.8a**	Colorado College	95	1.8 (P)	NA	NA	NA	NA	NA	P	NA	NO		NO
	1.8b	IUPUI	95	19.8	NA	NA	NA	NA	NA	NA	NA			
	1.8c	U. Havana	34	11.2	NA	NA	NA	NA	NA	NA	NA			
	1.8d	U. Havana	48	14.5	NA	NA	NA	NA	NA	NA	NA			
	**1.9a**	IUPUI	100	17.7	NA	NA	NA	NA	NA	NA	NA			
	1.9b	Goshen College	100	13.5	NA	NA	NA	NA	NA	NA	NA			
	**1.10a**	IUPUI	87	17.6	NA	NA	NA	NA	NA	NA	NA			
	1.10b	IUPUI	85	16.6	NA	NA	NA	NA	NA	NA	NA			
	**1.11a**	IUPUI	85 mol% (NMR)	9.5 (P)	NA	NA	NA	NA	NA	NA	NA			
	1.11b	IUPUI	84 mol% (NMR)	3.5 (P)	NA	NA	NA	NA	NA	NA	NA			
	1.11c	U. Havana	52	18.2	NA	NA	NA	NA	NA	NA	NA			
	1.11d	U. Havana	12	17.5	NA	NA	NA	NA	NA	NA	NA			
	**1.12a**	IUPUI	84	24.1	NA	NA	NA	NA	NA	NA	NA			
	1.12b	IUPUI	91	21.9	A	A	NA	P	A	NA	NA	NO		NO
	1.12c	U. Havana	76	15.8	NA	NA	NA	NA	NA	NA	NA			
	1.12d	U. Havana	10	14.7	NA	NA	NA	NA	NA	NA	NA			
	**1.13a**	U. Havana	97	16.0 10.8 (P)	NA	NA	NA	NA	NA	NA	NA			
	1.13b	U. Havana	94	20.0 10.8 (P)	NA	NA	NA	NA	NA	NA	NA			

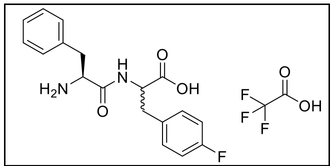
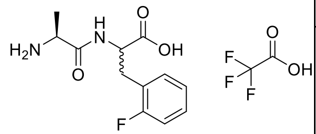
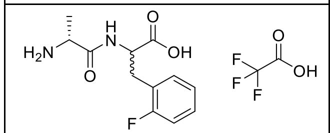
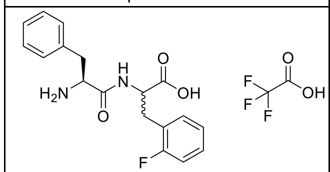
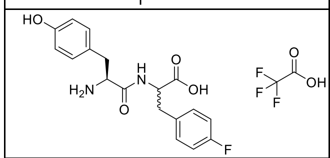
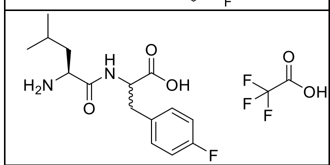
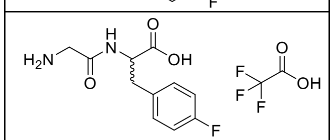
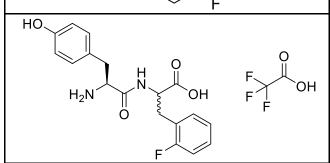
	**1.14a**	U. Havana	74	1.5	NA	NA	NA	NA	NA	NA	NA			
	1.14b	U. Havana	99	13.0	NA	NA	NA	NA	NA	NA	NA			
	**1.15a**	U. Havana	87	13.6	NA	NA	NA	NA	NA	NA	NA			
	1.15b	U. Havana	93	13.0	NA	NA	NA	NA	NA	NA	NA			
	**1.16a**	U. Havana	74	12.4	NA	NA	NA	NA	NA	NA	NA			
	1.16b	U. Havana	78	13.0	NA	NA	NA	NA	NA	NA	NA			
	**1.17a**	U. Havana	74	12.1	NA	NA	NA	NA	NA	NA	NA			
	1.17b	U. Havana	88	12.4	NA	NA	NA	NA	NA	NA	NA			
	**1.18a**	U. Havana	44	14.1	NA	NA	NA	NA	NA	NA	NA			
	1.18b	U. Havana	87	14.9	NA	NA	NA	NA	NA	NA	NA			
	**1.19a**	U. Havana	77	10.8	NA	NA	NA	NA	NA	NA	NA			
	1.19b	U. Havana	91	13.5	NA	NA	NA	NA	NA	NA	NA			
	**1.20a**	U. Havana	69	18.3	NA	NA	NA	NA	NA	NA	NA			
	1.20b	U. Havana	68	16.6	NA	NA	NA	NA	NA	NA	NA			
	**1.21a**	U. Havana	75	14.5	NA	NA	NA	NA	NA	NA	NA			
	1.21b	U. Havana	59	16.2	NA	NA	NA	NA	NA	NA	NA			

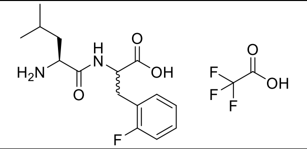
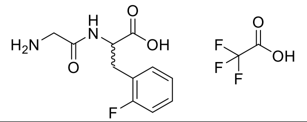
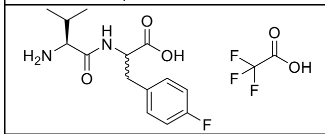
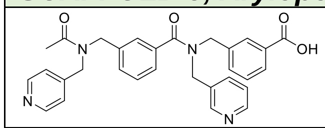
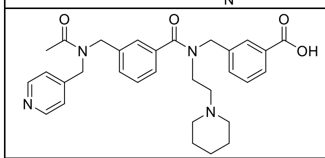
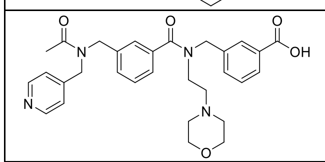
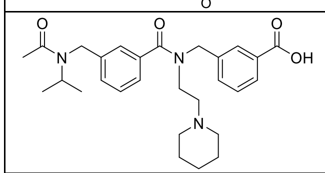
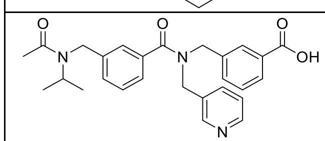
	**1.22a**	U. Havana	87	16.5	NA	NA	NA	NA	NA	NA	NA			
	1.22b	U. Havana	55	17.4	NA	NA	NA	NA	NA	NA	NA			
	**1.23a**	U. Havana	89	15.9	NA	NA	NA	NA	NA	NA	NA			
	1.23b	U. Havana	86	15.0	NA	NA	NA	NA	NA	NA	NA			
	**1.24a**	U. Havana	87	14.9	NA	NA	NA	NA	NA	NA	NA			
	1.24b	U. Havana	86	15.0	NA	NA	NA	NA	NA	NA	NA			
	**1.25a**	U. Havana	87	12.8	NA	NA	NA	NA	NA	NA	NA			
	1.25b	U. Havana	80	13.1	NA	NA	NA	NA	NA	NA	NA			
	**1.26a**	U. Havana	78	12.8	NA	NA	NA	NA	NA	NA	NA			
	1.26b	U. Havana	72	12.5	NA	NA	NA	NA	NA	NA	NA			
	**1.27**	Goshen	94	12.3	NA	NA	NA	NA	NA	NA	NA			
SCAFFOLD 2, N-Acylated unnatural amino acids														
	**2.1a**	IUPUI	100	19.0	NA	NA	NA	NA	NA	NA	NA			
	2.1b	IUPUI	100	17.0	NA	NA	NA	NA	P	NA	NA	NO		NO
	**2.2a**	IUPUI	88	15.5	NA	NA	NA	NA	P	NA	NA	NO		NO
	2.2b	IUPUI	100	16.6	NA	NA	NA	NA	NA	NA	NA			
	2.3a	IUPUI	90	17.0	NA	NA	NA	NA	NA	NA	NA			

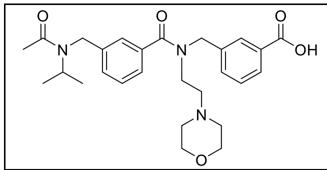
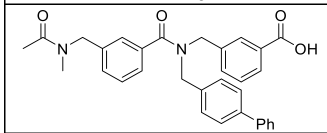
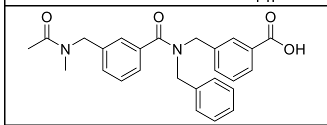
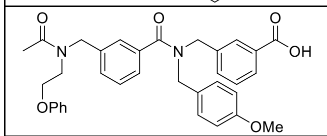
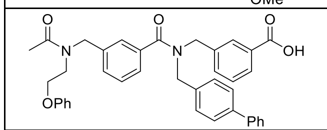
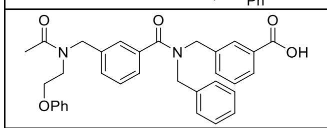
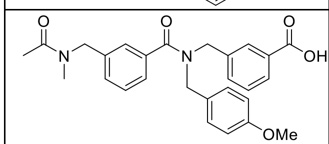
	**2.3b**	IUPUI	88	16.5	NA	NA	NA	NA	NA	NA	NA			
	**2.4a**	IUPUI	91	18.6	NA	NA	NA	NA	NA	NA	NA			
	2.4b	IUPUI	86	20.8	NA	NA	NA	NA	NA	NA	NA			
	**2.5a**	IUPUI	86	14.9	NA	NA	NA	NA	NA	NA	NA			
	2.5b	IUPUI	88	16.0	NA	NA	NA	NA	NA	NA	NA			
	**2.6a**	IUPUI	90	7.4	NA	NA	NA	NA	NA	NA	NA			
	2.6b	IUPUI	95	17.8	NA	NA	NA	NA	NA	NA	NA			
	**2.7a**	IUPUI	88	21.0	NA	NA	NA	NA	NA	NA	NA			
	2.7b	IUPUI	94	14.0	NA	NA	NA	NA	P	NA	NA	NO		NO
	**2.8a**	IUPUI	81	20.3	NA	NA	NA	NA	P	NA	NA	NO		NO
	2.8b	IUPUI	86	20.6	NA	NA	NA	NA	P	NA	NA	NO		NO
	**2.9a**	IUPUI	89	18.4	NA	NA	NA	NA	P	NA	NA	NO		NO
	2.9b	IUPUI	93	16.4	NA	NA	NA	NA	NA	NA	NA			
	**2.10a**	IUPUI	92	14.8	NA	NA	NA	NA	NA	NA	NA			
	2.10b	IUPUI	92	17.8	NA	NA	NA	NA	NA	NA	NA			
	2.11a	IUPUI	90	25.0	NA	NA	NA	NA	NA	NA	NA			

	**2.11b**	IUPUI	89	35.0	NA	NA	NA	NA	NA	NA	NA			
	**2.12a**	IUPUI	88	13.0	NA	NA	NA	NA	NA	NA	NA			
	2.12b	IUPUI	83	17.0	NA	NA	NA	NA	NA	NA	NA			
SCAFFOLD 3, Unnatural α-amino acid amides														
	**3.1a**	IUPUI	100	8.4	NA	NA	NA	NA	NA	NA	NA			
	3.1b	IUPUI	100	7.4	NA	NA	NA	NA	P	NA	NA	NO		NO
	**3.2a**	IUPUI	100	7.3	NA	NA	NA	NA	P	NA	NA	NO		NO
	3.2b	IUPUI	100	6.1	NA	NA	NA	NA	P	NA	NA	NO		NO
	**3.3a**	IUPUI	96	9.0	NA	NA	NA	NA	NA	NA	NA			
	3.3b	IUPUI	97	7.4	NA	NA	NA	NA	NA	NA	NA			
	**3.4a**	IUPUI	92	9.5	NA	NA	NA	NA	NA	NA	NA			
	3.4b	IUPUI	92	9.5	NA	NA	NA	NA	NA	NA	P	NO		NO
	**3.5a**	IUPUI	100	8.4	NA	NA	NA	NA	NA	NA	NA			
	3.5b	IUPUI	100	7.2	NA	NA	NA	NA	NA	NA	NA			
	**3.6a**	IUPUI	96	9.4	NA	NA	NA	NA	P	NA	NA	NO		NO
	3.6b	IUPUI	100	8.8	NA	NA	NA	NA	NA	NA	NA			
	3.7a	IUPUI	89	11.0	NA	NA	NA	NA	NA	NA	NA			

	**3.7b**	IUPUI	100	9.1	NA	NA	NA	NA	NA	NA	NA			
	**3.8a**	IUPUI	98	13.2	NA	NA	NA	NA	NA	NA	NA			
	3.8b	IUPUI	95	12.5	NA	NA	NA	NA	P	NA	NA	NO		NO
	**3.9a**	IUPUI	80	Not obtained	NA	NA	NA	NA	NA	NA	NA			
	3.9b	IUPUI	90	9.4	NA	NA	NA	NA	NA	NA	NA			
	**3.10a**	IUPUI	100	9.6	NA	NA	NA	NA	NA	A	P	NO		NO
	3.10b	IUPUI	100	12	NA	NA	NA	NA	NA	A	A	NO		NO
	**3.11a**	IUPUI	100	11.7	NA	NA	NA	NA	NA	NA	P	NO		NO
	3.11b	IUPUI	100	10.0	NA	NA	NA	NA	NA	NA	NA			
	**3.12a**	IUPUI	92	7.8	NA	NA	NA	NA	NA	NA	NA			
	3.12b	IUPUI	100	7.9	NA	NA	NA	NA	NA	NA	P	NO		NO
SCAFFOLD 4, Dipeptides with an unnatural amino acid R[1]														
	**4.1a**	IUPUI	76	19.0 7.5 (P)	NA	NA	NA	NA	NA	NA	A	YES	*C. neoformans*, 8	NO
	4.1b	IUPUI	86	19.8	NA	NA	NA	NA	NA	NA	P	YES	*C. neoformans*, 4	NO
	**4.2a**	IUPUI	86	16.6	NA	NA	NA	NA	NA	NA	NA			
	4.2b	IUPUI	84	12.7	NA	NA	NA	NA	NA	NA	NA			
	4.3a	IUPUI	80	19.7	NA	NA	NA	NA	NA	NA	NA			

	**4.3b**	IUPUI	76	16.5	NA	NA	NA	NA	NA	NA	NA			
	**4.4a**	IUPUI	85	15.8	NA	NA	NA	NA	NA	NA	A	YES	*C. neoformans*, 8	NO
	4.4b	IUPUI	83	19.2	NA	NA	NA	NA	NA	NA	A	YES	*C. neoformans*, 8	NO
	**4.5a**	IUPUI	85	16.3	NA	NA	NA	NA	NA	NA	NA			
	4.5b	IUPUI	89	20.9	NA	NA	NA	NA	NA	NA	NA			
	**4.6a**	IUPUI	82	19.2	NA	NA	NA	NA	NA	NA	NA			
	4.6b	IUPUI	82	23.6	NA	NA	NA	NA	NA	NA	NA			
	**4.7a**	IUPUI	75	24.1	NA	NA	NA	NA	NA	NA	A	NO		NO
	4.7b	IUPUI	72	16.9	NA	NA	NA	NA	NA	NA	A	NO		NO
	**4.8a**	IUPUI	89	20.0	NA	NA	NA	NA	P	NA	NA	NO		YES
	4.8b	IUPUI	74	15.5	NA	NA	NA	NA	P	NA	NA	NO		NO
	**4.9a**	IUPUI	92	23.3	NA	NA	NA	NA	NA	NA	A	NO		YES
	4.9b	IUPUI	68	10.5	NA	NA	NA	NA	NA	NA	A	NO		NO
	**4.10a**	IUPUI	80	25.3	NA	NA	NA	NA	NA	NA	NA			
	4.10b	IUPUI	81	16.9	NA	NA	NA	NA	P	NA	NA	NO		NO
	4.11a	IUPUI	90	23.8	NA	NA	NA	NA	P	P	NA	NO		NO

	**4.11b**	IUPUI	75	14.5	NA	NA	NA	NA	NA	P	NA	NO		NO
	**4.12**	IUPUI	87	21.5	NA	NA	NA	NA	NA	NA	A	NO		NO
	**4.13**	IUPUI	74	21.2	NA	NA	NA	NA	NA	NA	A	NO		NO
SCAFFOLD 5, Arylopeptoids														
	**5.1a**	IUPUI	100	14.0 2.0 (P)	NA	NA	NA	NA	NA	NA	NA			
	5.1b	Santa Clara	82	12.9	NA	NA	NA	NA	NA	NA	NA			
	**5.2a**	IUPUI	100	17.5 4.5 (P)	NA	NA	NA	NA	NA	NA	NA			
	5.2b	Santa Clara	73	17.3	NA	NA	NA	NA	NA	NA	NA			
	**5.3a**	IUPUI	100	17.8 5.1 (P)	NA	NA	NA	NA	NA	NA	NA			
	5.3b	Santa Clara	67	18.4	NA	NA	NA	NA	NA	NA	NA			
	**5.4a**	IUPUI	100	14.3 4.6 (P)	NA	NA	NA	NA	NA	NA	P	NO		NO
	5.4b	Santa Clara	77	16.4	NA	NA	NA	NA	NA	NA	P	NO		NO
	**5.5a**	IUPUI	100	12.5 2.5 (P)	NA	NA	NA	NA	NA	NA	NA			
	5.5b	Santa Clara	84	14.6	NA	NA	NA	NA	NA	NA	NA			
	5.6a	IUPUI	100	19.2 4.3 (P)	NA	NA	NA	NA	NA	NA	NA			

	**5.6b**	Santa Clara	67	7.8	NA	NA	NA	NA	NA	NA	NA			
	**5.7a**	IUPUI	100	13.2 1.8 (P)	NA	NA	NA	NA	NA	NA	NA			
	5.7b	Santa Clara	89	10.8 6.5 (P)	NA	NA	NA	NA	NA	NA	NA			
	**5.8a**	IUPUI	100	15.4 0.8 (P)	NA	NA	NA	NA	NA	NA	NA			
	5.8b	Santa Clara	100	9.4 3.9 (P)	NA	NA	NA	NA	NA	NA	NA			
	**5.9a**	IUPUI	100	Not obtained	NA	NA	NA	NA	NA	NA	NA			
	5.9b	Santa Clara	91	13.8 10.2 (P)	NA	NA	NA	NA	NA	NA	NA			
	**5.10a**	IUPUI	100	17.2 3.5 (P)	NA	NA	NA	NA	NA	NA	NA			
	5.10b	Santa Clara	88	12.1 8.4 (P)	NA	NA	NA	NA	NA	NA	NA			
	**5.11a**	IUPUI	100	15.4 4.7 (P)	NA	NA	NA	NA	NA	NA	NA			
	5.11b	Santa Clara	88	10.8 8.4 (P)	NA	NA	NA	NA	NA	NA	NA			
	**5.12a**	IUPUI	100	15.3 1.2 (P)	NA	NA	NA	NA	NA	NA	NA			
	5.12b	Santa Clara	100	9.5 7.7 (P)	NA	NA	NA	NA	NA	NA	NA			

[a] **Quantitative NMR Analyses (QNMR):** Approximately 150 μL hexamethyldisiloxane (HMDS) was added to approximately 100 mL of $CDCl_3$. This solution was then standardized using dimethylterephthalate (DMTP) according to the following procedure: Dimethyl terephthalate (0.9709 g, 5.000 mmol) was added to a 100 mL volumetric flask and dissolved in CH_3CN to give a final concentration of 0.0500 M. Three separate 1.00 mL aliquots (50.0 μmol each) of this solution were evaporated to dryness. Each residue was then dissolved in 1.00 mL of the hexamethyldisoloxane solution in $CDCl_3$ and the solutions were then transferred to individual NMR tubes and spectra obtained referenced by HMDS (0.065 ppm) and integration performed. Integration values for the methyl groups of DMTP were chosen for the standardization calculations. Calculations were performed using Equations **1** and **2**. Equation **1** equates the ratio of HMDS and DMTP with the ratio demonstrated by ^{1}HNMR.

$$\frac{\text{moles of HMDS}}{\text{moles of DMTP}} = \frac{\dfrac{\text{integration units of peak A(HMDS)}}{\text{protons of peak A(HMDS)}}}{\dfrac{\text{integration units of peak B(DMTP)}}{\text{protons of peak B(DMTP)}}} \qquad \textbf{(1)}$$

$$\text{moles of DMTP} \cdot \frac{\text{number of DMTP protons}}{\text{integration of DMTP peak}} \cdot \frac{\text{HMDS integration units}}{\text{18 HMDS protons}} = \text{moles of HMDS} \qquad \textbf{(2)}$$

The molarity of the HMDS solution in $CDCl_3$ is then found by dividing the moles of HMDS by 0.00100 L. The three molarity results were then averaged.

Quantitative analysis of crude reaction products was performed in an identical manner utilizing a signal in the crude NMR spectrum known to arise solely from the component undergoing analysis. Values for the terms associated with DMTP in the equations **1** and **2** are replaced with those associated with the component undergoing analysis. All values for the terms associated with HMDS are known.

For chloroform insoluble products methanol-d_4 was used as an NMR co-solvent. The amount of HMDS in $CDCl_3$ used was 0.30 mL.